Memorial Sloan Kettering
Cancer Center

CYTEK® Aurora
Creating an Experiment

Contents:

1. Setting up a new experiment
2. Adjusting User Settings
3. Recording Controls & Unmixing
4. Recording Experimental Samples
5. Data Export
6. Cleaning

Flow Cytometry
Core Facility

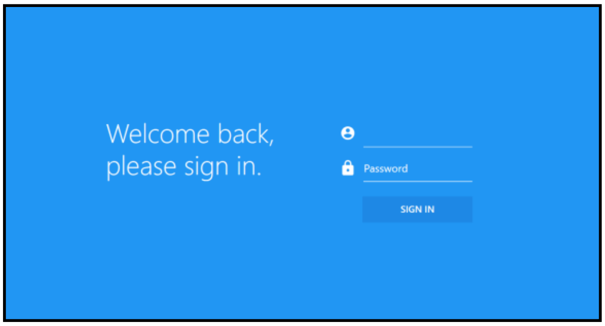
Ver. 3
2022-10-10

1. Setting up a new experiment

1) Launch SpectroFLO Software.

Login name:

Password:

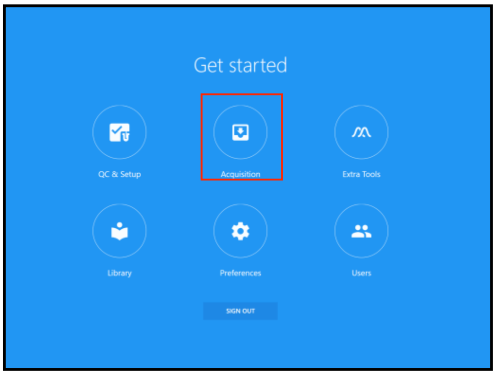


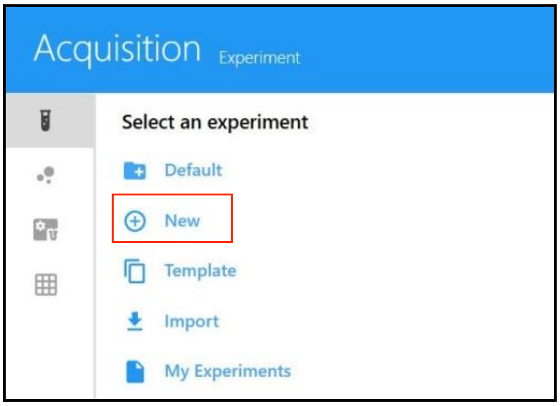
Welcome back, please sign in.

2) If you are the first user of the day, follow startup instructions as per **CYTEK® Aurora Startup, QC, Cleaning & Shutdown Procedure**.

3) Select Acquisition from the Get started screen.

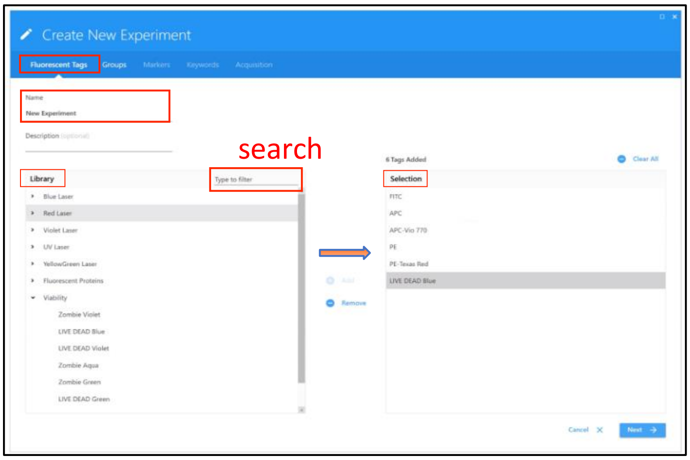
4) Create a new experiment.





5) Fluorescent Tags Tab

- Rename your experiment
- From the fluorochrome library, search for the fluorochromes in your panel. Double click or highlight and choose **add** to include it in your experiment.
- To confirm the selected list, click **Next**.



Name: New Experiment

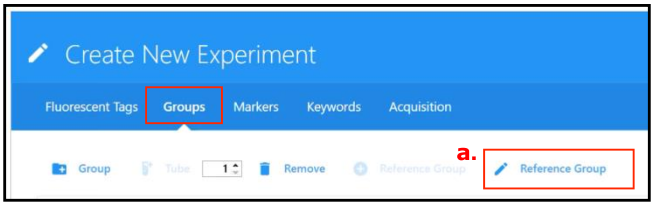
Description (optional)

6 Tags Added

Selection:
FITC
APC
APC-Vio 770
PE
PE-Texas Red
LIVE DEAD Blue

6) Create Reference Group

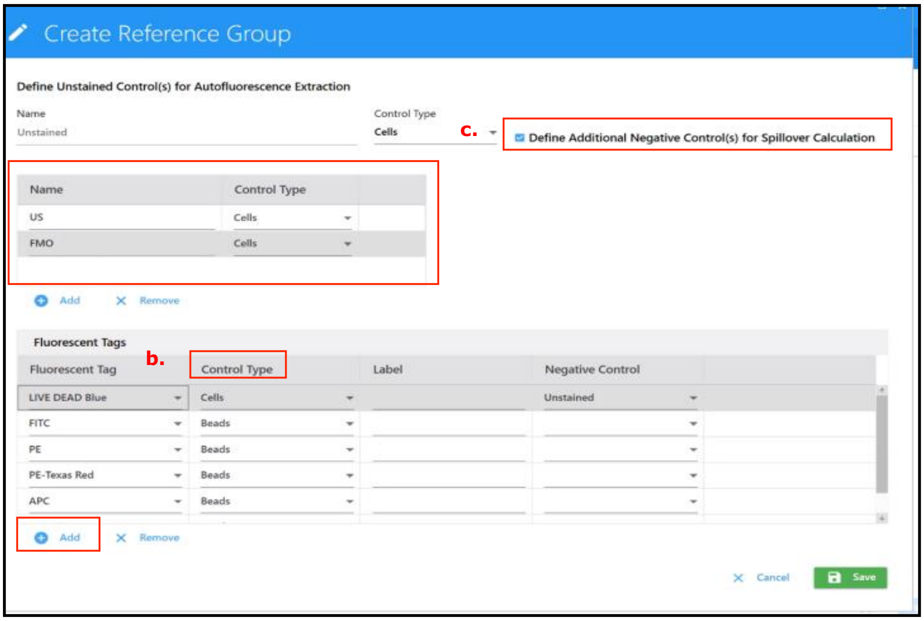
- From the Groups select reference group (a).



- SpectroFlo automatically adds an unstained control and single color controls for each fluorochrome in your experiment.

Note: An unstained sample of the same cell type as your experimental samples must be run as it is a part of the unmixing process. **Control type (b)** (beads or cells) must be selected from the dropdown menu.

- If using a mix of beads and cells or different types of cells - additional unstained controls can be added **(c).**
- Hit **Save** to confirm defined controls.



Define Unstained Control(s) for Autofluorescence Extraction

Name: Unstained

Control Type: Cells

☑ Define Additional Negative Control(s) for Spillover Calculation

Name	Control Type
US	Cells
FMO	Cells

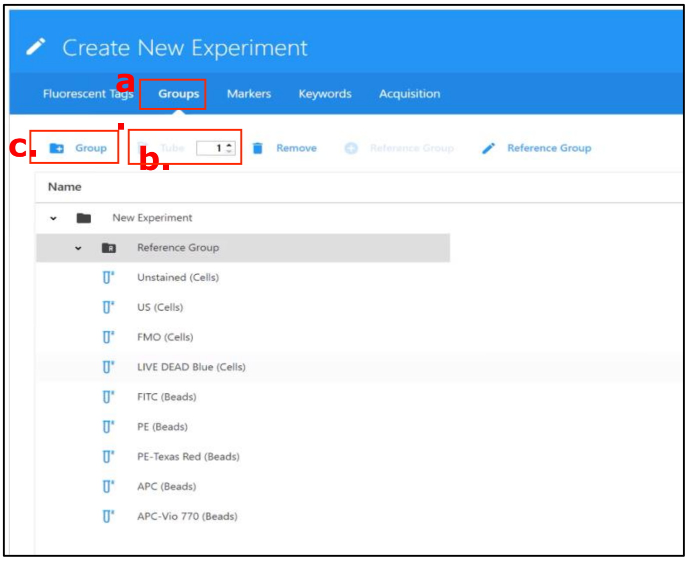
Fluorescent Tags

Fluorescent Tag	Control Type	Label	Negative Control
LIVE DEAD Blue	Cells		Unstained
FITC	Beads		
PE	Beads		
PE-Texas Red	Beads		
APC	Beads		

7) Create Experimental Sample Groups

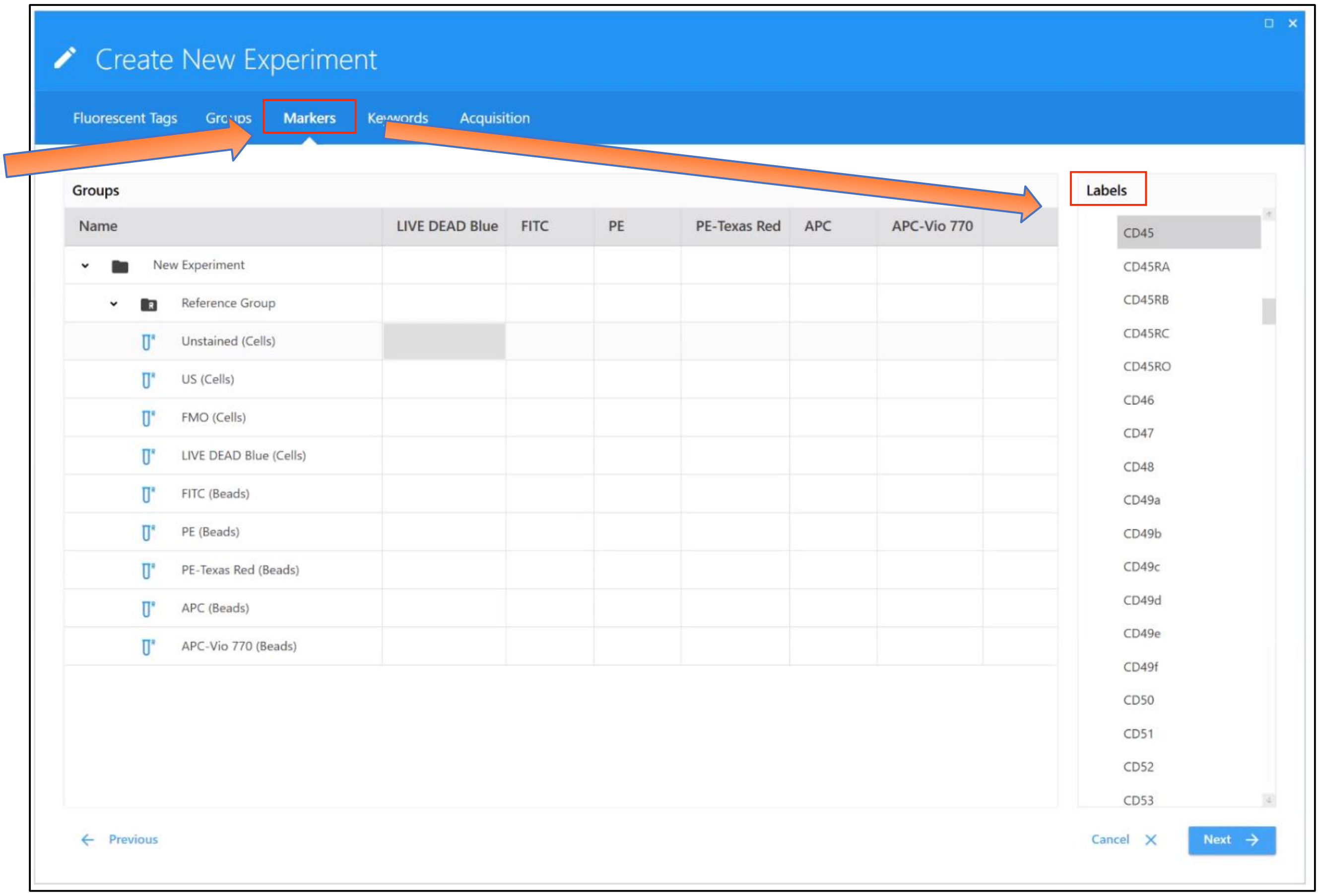
- To add an **experimental sample group**, from the **Groups (a)** tab, enter the number of tubes **(b)** you would like to add in the group, and select + **Group** icon **(c).**
- If there are different conditions (e.g., different tissue, different cell lines etc.) multiple groups can be created.

Note: Group names won't be saved into FCS files.



8) Markers – labeling

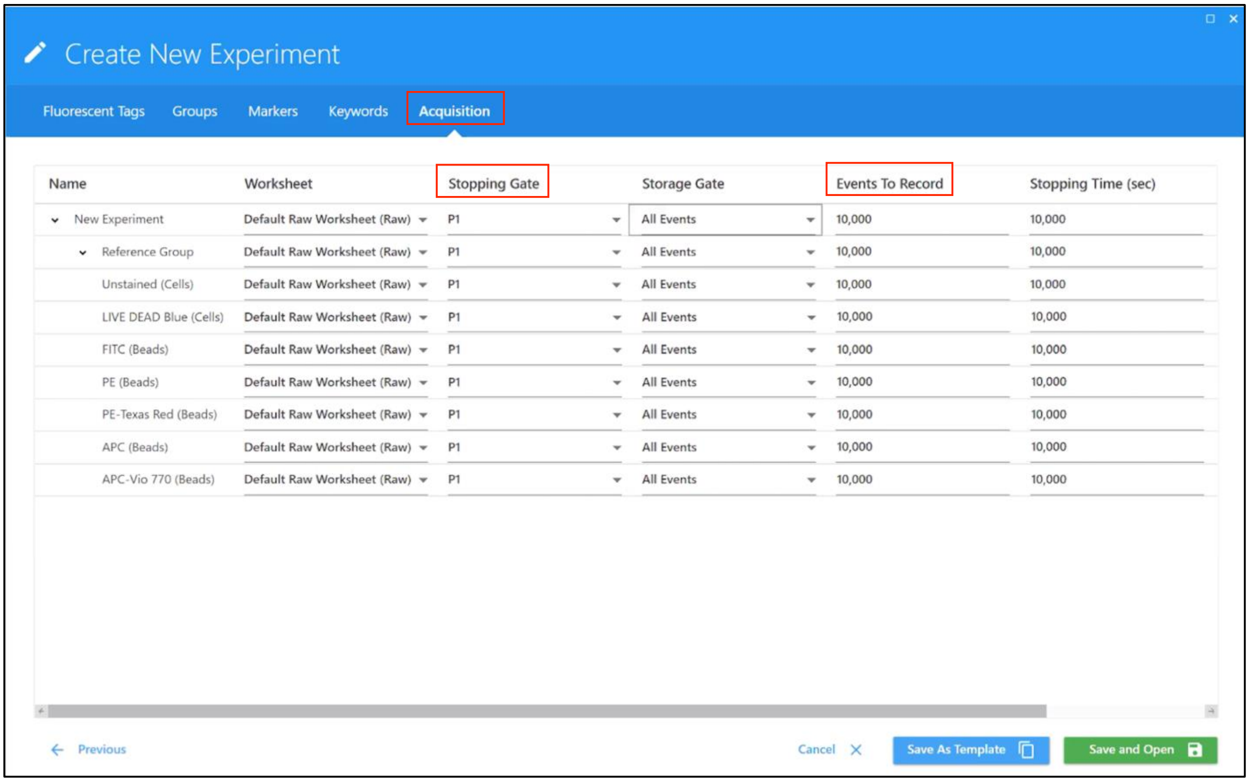
- From the "Markers" tab, set labels for each of your fluorescent tags by double clicking on the text box underneath the fluorochrome name and typing in your desired label. Select **enter** on the keyboard (note: you can have group/tube specific labels).
- When you are done labeling, click **Next.**



2. Adjusting User Settings

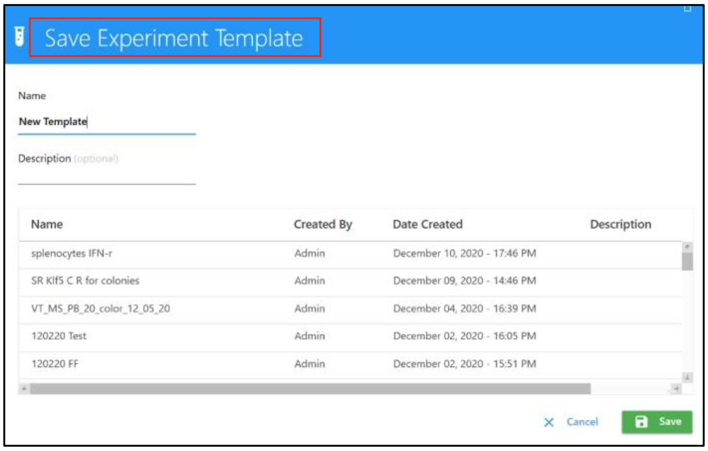
1) Acquisition Tab:

- At this stage you can define and set up the worksheet, stopping gates, events to record for references controls and experimental samples (these options can be modified at any point).
- For reference group samples, always work from the default raw worksheet. **Stopping gate** can be set to **P1 10,000 events for beads, or P1 20,000 events for cell controls**. For cellular reference controls with rare expression, ensure that at least **400 - 1000 positive events** are recorded. Storage gate is not suggested, as it will not record anything that doesn't meet that criteria.



Name	Worksheet	Stopping Gate	Storage Gate	Events To Record	Stopping Time (sec)
New Experiment	Default Raw Worksheet (Raw)	P1	All Events	10,000	10,000
Reference Group	Default Raw Worksheet (Raw)	P1	All Events	10,000	10,000
Unstained (Cells)	Default Raw Worksheet (Raw)	P1	All Events	10,000	10,000
LIVE DEAD Blue (Cells)	Default Raw Worksheet (Raw)	P1	All Events	10,000	10,000
FITC (Beads)	Default Raw Worksheet (Raw)	P1	All Events	10,000	10,000
PE (Beads)	Default Raw Worksheet (Raw)	P1	All Events	10,000	10,000
PE-Texas Red (Beads)	Default Raw Worksheet (Raw)	P1	All Events	10,000	10,000
APC (Beads)	Default Raw Worksheet (Raw)	P1	All Events	10,000	10,000
APC-Vio 770 (Beads)	Default Raw Worksheet (Raw)	P1	All Events	10,000	10,000

- **Save and open** if you want to run the experiment or **save as template.**
- Experimental templates will save to the library. These templates will be available for any future experimental runs.



Name
New Template

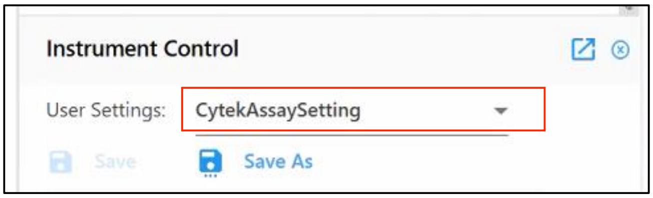
Description (optional)

Name	Created By	Date Created	Description
splenocytes IFN-r	Admin	December 10, 2020 - 17:46 PM	
SR Klf5 C R for colonies	Admin	December 09, 2020 - 14:46 PM	
VT_MS_PB_20_color_12_05_20	Admin	December 04, 2020 - 16:39 PM	
120220 Test	Admin	December 02, 2020 - 16:05 PM	
120220 FF	Admin	December 02, 2020 - 15:51 PM	

3. Recording Controls

1) After selecting **save and open,** navigate to the *Instrument Control Tab (left bottom side)* and choose “CytekAssaySetting” for the starting User Settings.

- Importantly, "CytekAssaySetting" are the optimized starting gains for all detectors on the Aurora. Changes should only be made to bring cells on scale by scatter or to bring fluorescent signal on scale.



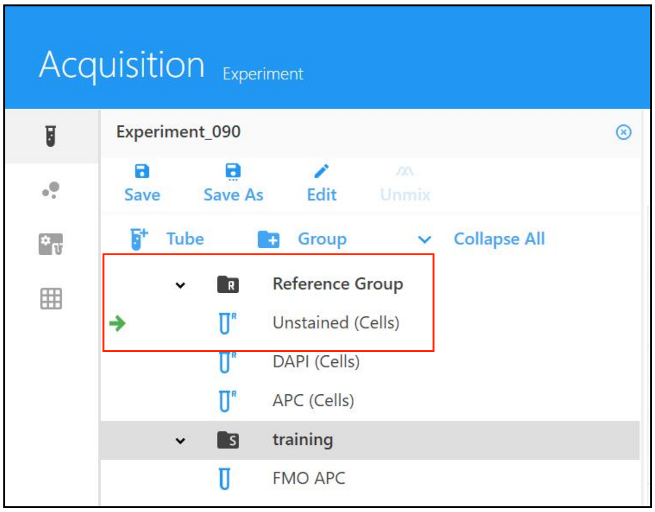
Instrument Control

User Settings: CytekAssaySetting

2) Load an unstained sample on the loading station and click **Start** in the *Acquisition Control Window*. By clicking on the *Flow Rate* window, you can adjust the acquisition speed and the number of events to display. When you first load the sample, keep the flow rate at **low** and monitor event rate.

- While Cytek says the instrument can process 30k events per second, we advise to monitor the abort rate and keep it **under 5%** (for example: if the flow rate is 10.000/sec, the abort rate should not be higher than 500).

- The unstained cell sample from reference group should be selected in the sample list (green arrow).

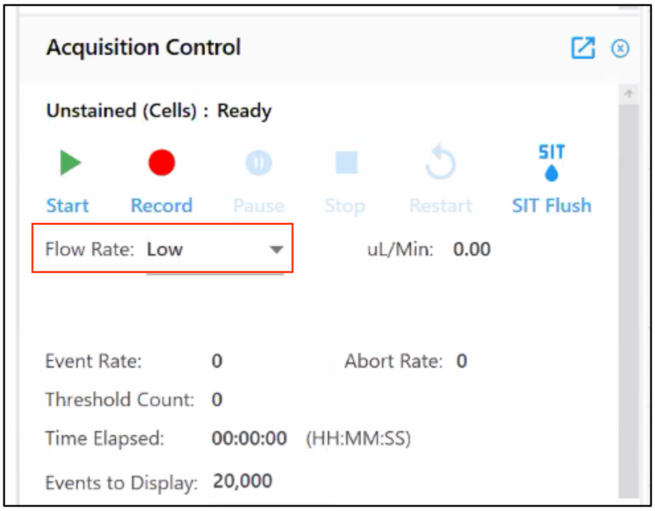


Acquisition Experiment

Experiment_090

Reference Group
- Unstained (Cells)
- DAPI (Cells)
- APC (Cells)

training
- FMO APC



Acquisition Control

Unstained (Cells) : Ready

Flow Rate: Low

uL/Min: 0.00

Event Rate: 0

Abort Rate: 0

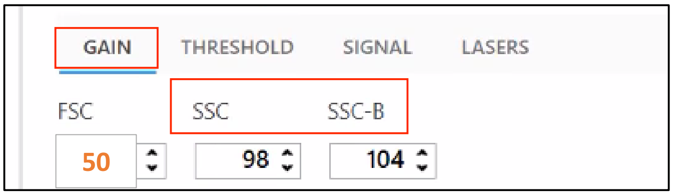
Threshold Count: 0

Time Elapsed: 00:00:00 (HH:MM:SS)

Events to Display: 20,000

3) While running your unstained sample, (Instrument Control Window), adjust gains and the threshold value. Notably, with the unstained sample the scatter settings are adjusted to bring the cells on scale.

- The FSC and SSC settings from the Cytekassaysettings are optimized for the Spectroflo beads. Thus, reduce the FSC to bring cells on scale - typically, a **good starting point is 50**.

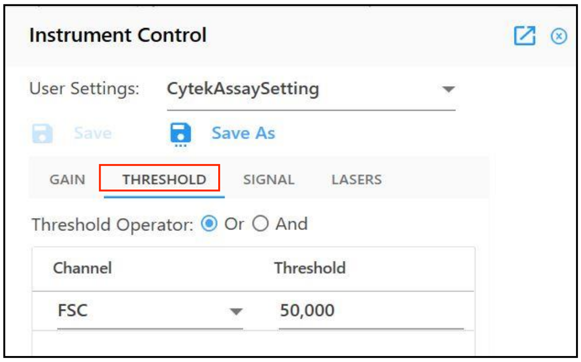

GAIN THRESHOLD SIGNAL LASERS

FSC: 50

SSC: 98

SSC-B: 104

- Please be aware that SSC by default is off the violet laser and that SSC-B is off the blue laser. Either can be used, but the violet SSC is preferred for submicron flow cytometry.
- A threshold that is too high may prevent the cytometer from displaying cells of interest (dying/dead or smaller cells in heterogenous populations).

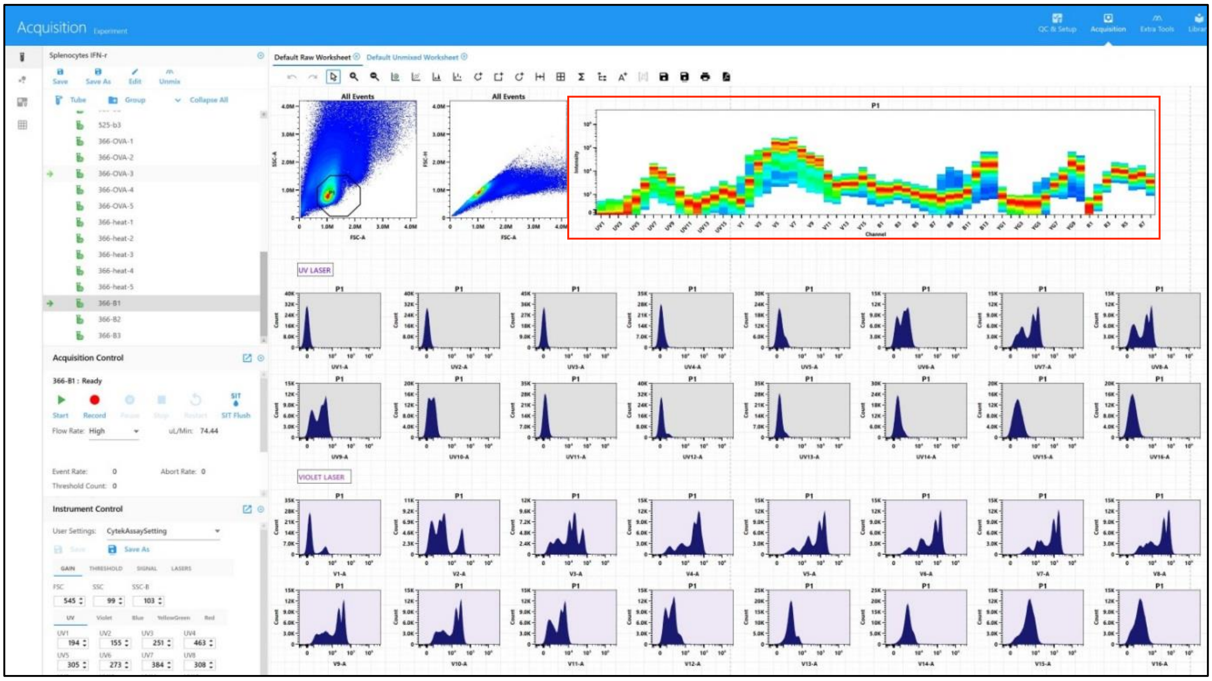

Instrument Control

User Settings: CytekAssaySetting

Threshold Operator: ● Or ○ And

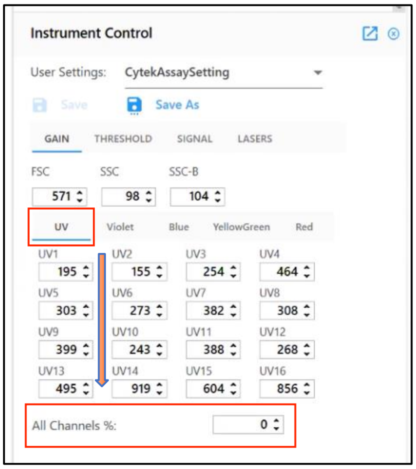
Channel	Threshold
FSC	50,000

4) After the unstained control, run a small aliquot of an experimental sample, which is expected to have the brightest signal. Check the full spectrum plot to ensure that all signal is on scale.



- If any data is off scale for fluorescence, **never adjust that individual detector, but instead reduce the gain for "all channels%" of that laser**. Gain reduction is % based and should only be reduced for all laser lines until the signal is on scale.

- When all parameters are set properly, Reference Controls are ready for acquisition. Therefore, click **Start** and **Record** for each sample from the reference group.

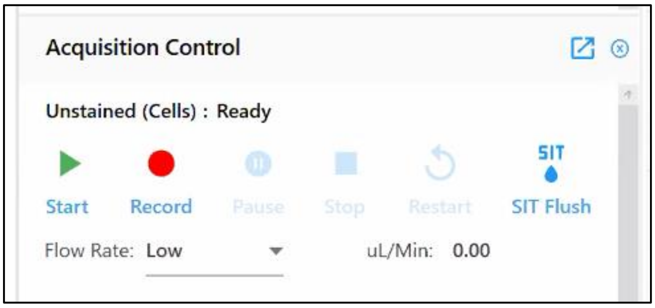

Instrument Control

User Settings: CytekAssaySetting

FSC 571
SSC 98
SSC-B 104

UV1	UV2	UV3	UV4
195	155	254	464
UV5	UV6	UV7	UV8
303	273	382	308
UV9	UV10	UV11	UV12
399	243	388	268
UV13	UV14	UV15	UV16
495	919	604	856

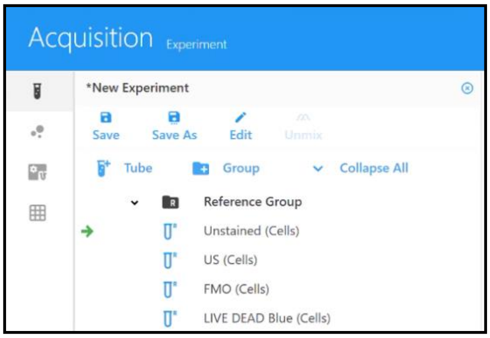
All Channels %: 0


Acquisition Control

Unstained (Cells) : Ready

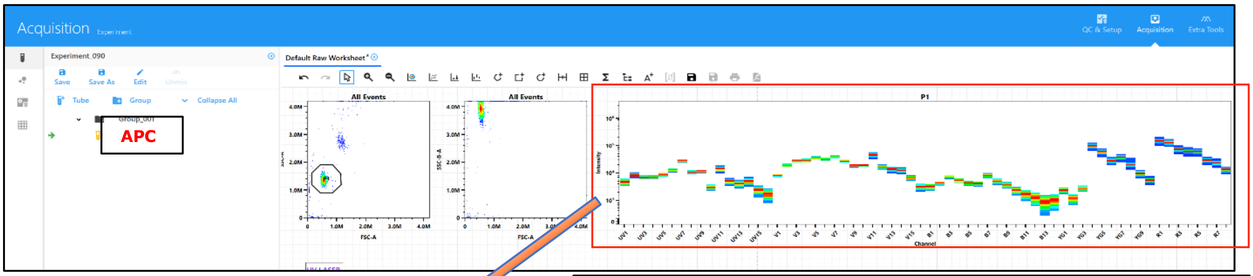
Flow Rate: Low

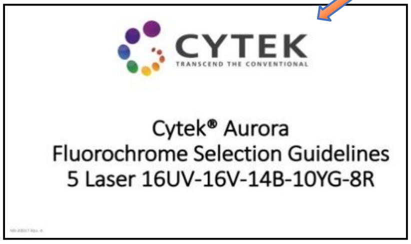
uL/Min: 0.00



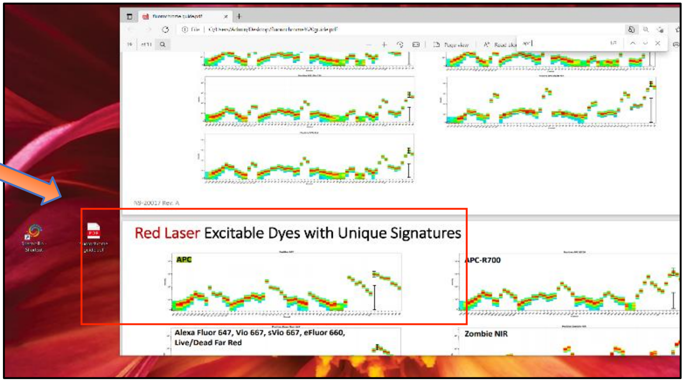
5) Check each reference control to ensure that the full spectrum signature matches what is expected. Compare the signature from the full spectrum plot in SpectroFlo with an image from the [Cytek's fluorochrome guide:](#)

- If a fluorochrome is not listed on Cytek's fluorochrome guide, please search for the spectra from spectrum.cytekbio.com and remember to select 5L configuration.




CYTEK
TRANSCEND THE CONVENTIONAL

Cytek® Aurora
Fluorochrome Selection Guidelines
5 Laser 16UV-16V-14B-10YG-8R


Red Laser Excitable Dyes with Unique Signatures

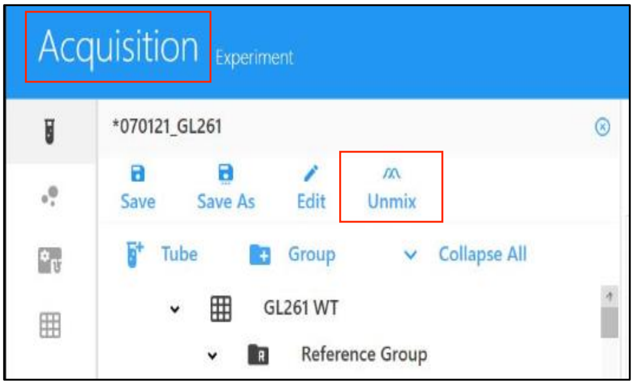
APC
APC-R700
Alexa Fluor 647, Vio 667, sVio 667, eFluor 660, Live/Dead Far Red
Zombie NIR

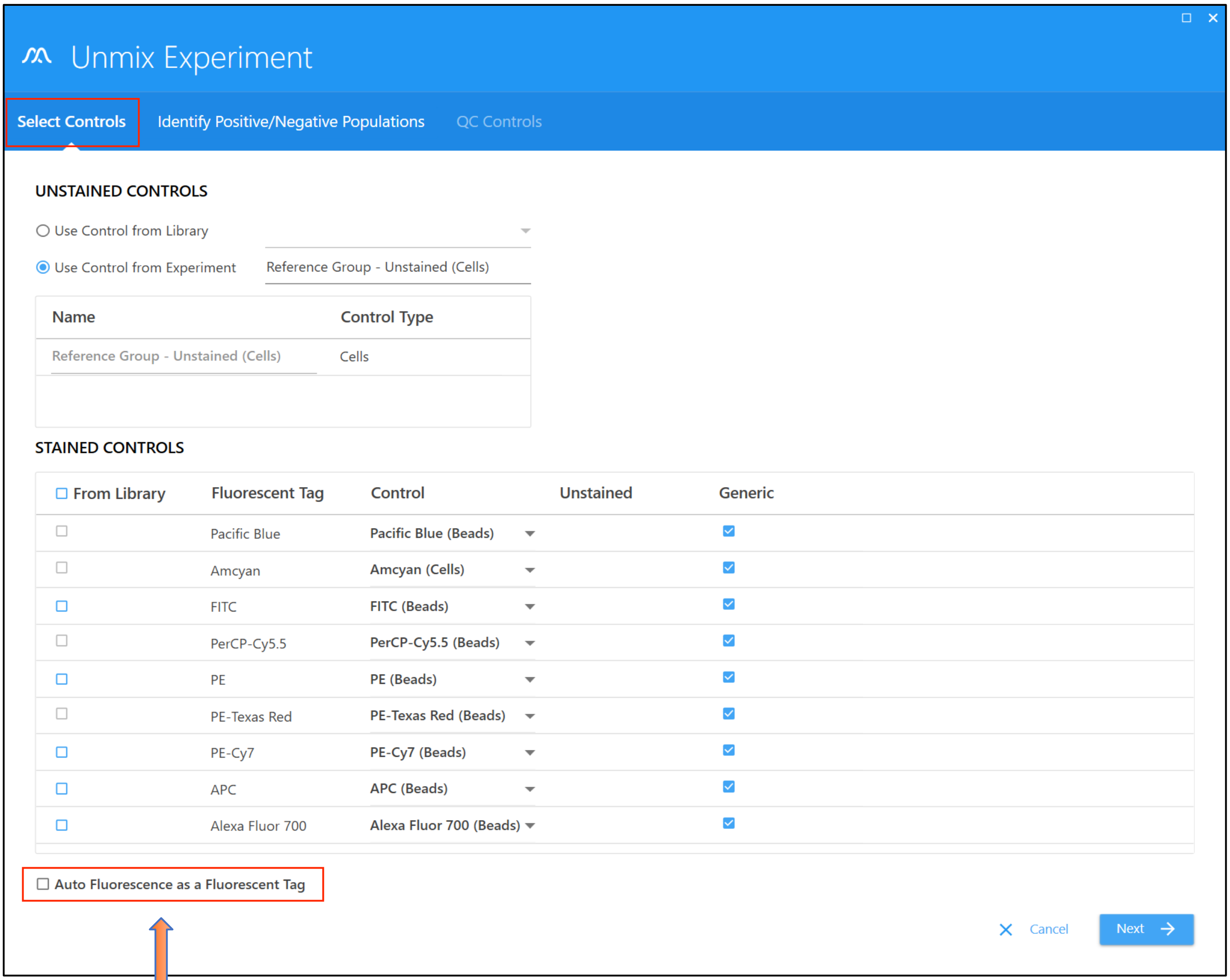
6) After all reference group samples are recorded, proceed to unmixing.

4. Unmixing

1) From the Acquisition Control Window click on unmix.

2) After selecting unmixing, you will have to make sure to select controls.





UNSTAINED CONTROLS

○ Use Control from Library

● Use Control from Experiment — Reference Group - Unstained (Cells)

Name	Control Type
Reference Group - Unstained (Cells)	Cells

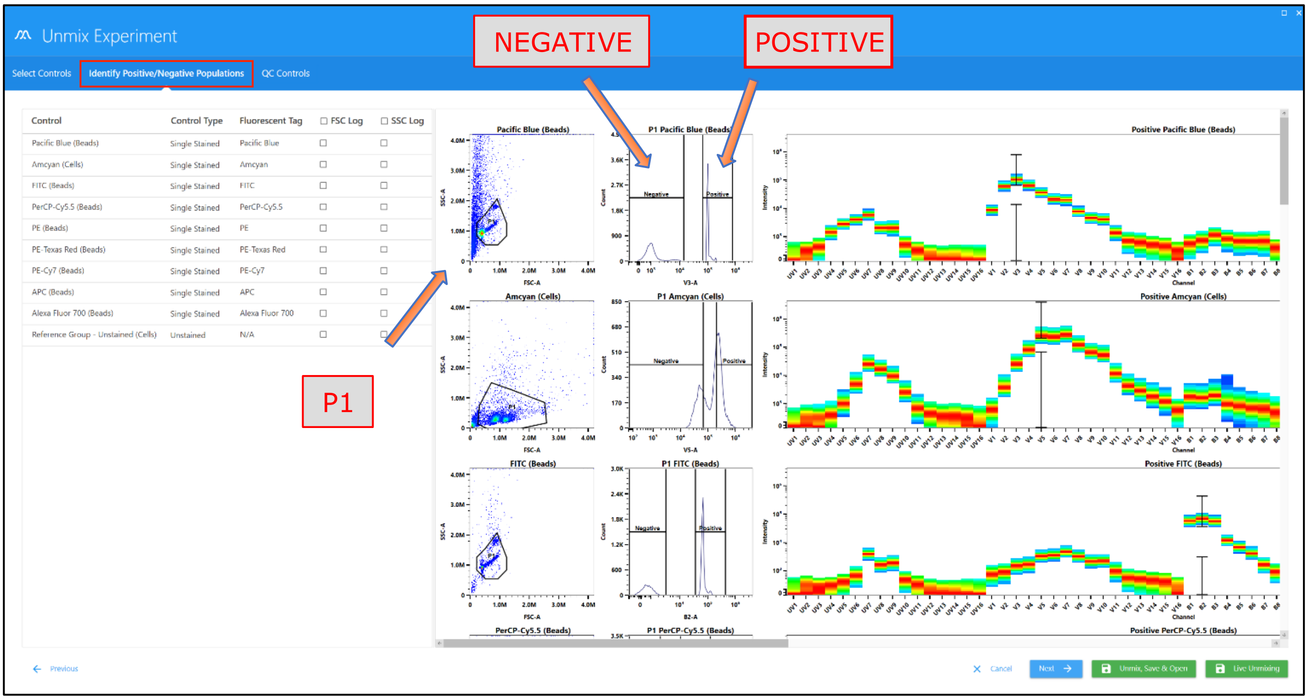
STAINED CONTROLS

☐ From Library	Fluorescent Tag	Control	Unstained	Generic
☐	Pacific Blue	Pacific Blue (Beads)		☑
☐	Amcyan	Amcyan (Cells)		☑
☐	FITC	FITC (Beads)		☑
☐	PerCP-Cy5.5	PerCP-Cy5.5 (Beads)		☑
☐	PE	PE (Beads)		☑
☐	PE-Texas Red	PE-Texas Red (Beads)		☑
☐	PE-Cy7	PE-Cy7 (Beads)		☑
☐	APC	APC (Beads)		☑
☐	Alexa Fluor 700	Alexa Fluor 700 (Beads)		☑

☐ Auto Fluorescence as a Fluorescent Tag

- Cytek Aurora allows to extract a sample's auto fluorescence and treat it as a separate parameter, which can improve the resolution for specific cell types. Therefore, you can click the box “Auto Fluorescence as a Fluorescent Tag” and click on Next.

3) After selecting all controls, identify an appropriate **P1 gate and positive and negative populations for each single color control. Note:** negative population is automatically set based on previously defined unstained control



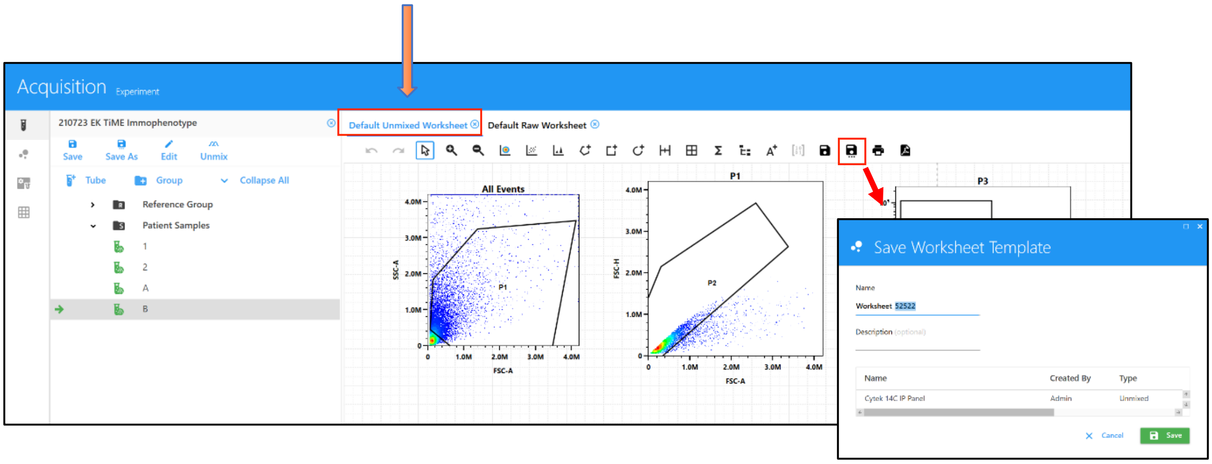
Control	Control Type	Fluorescent Tag	☐ FSC Log	☐ SSC Log
Pacific Blue (Beads)	Single Stained	Pacific Blue	☐	☐
Amcyan (Cells)	Single Stained	Amcyan	☐	☐
FITC (Beads)	Single Stained	FITC	☐	☐
PerCP-Cy5.5 (Beads)	Single Stained	PerCP-Cy5.5	☐	☐
PE (Beads)	Single Stained	PE	☐	☐
PE-Texas Red (Beads)	Single Stained	PE-Texas Red	☐	☐
PE-Cy7 (Beads)	Single Stained	PE-Cy7	☐	☐
APC (Beads)	Single Stained	APC	☐	☐
Alexa Fluor 700 (Beads)	Single Stained	Alexa Fluor 700	☐	☐
Reference Group - Unstained (Cells)	Unstained	N/A	☐	☐

- Once finished, you can hit either on **Live Unmixing or Unmix, Save & Open**.

*We advise you to carry out Live Unmixing, so that all the reference samples (single color), which have been already run are unmixed and the unmixing matrix will be applied to your experimental samples during acquisition. Therefore, you will have a better estimation whether unmixing works and if all your controls are correct. If you are looking at data that has been already recorded, you can run Unmix, Save & Open. It will open a new experiment and apply the unmixing to it. However, in the new experiment you will have only unmixed data, you will not see the default raw worksheet. Unmix, Save & Open allows you to import an experiment to another Aurora instrument (even if configurations do not match.)

5. Recording Experimental Samples

1) After unmixing, a new "**Default Unmixed Worksheet**" tab is shown in the acquisition tab. Please never overwrite data in this worksheet, but instead *Save as* under a different name.

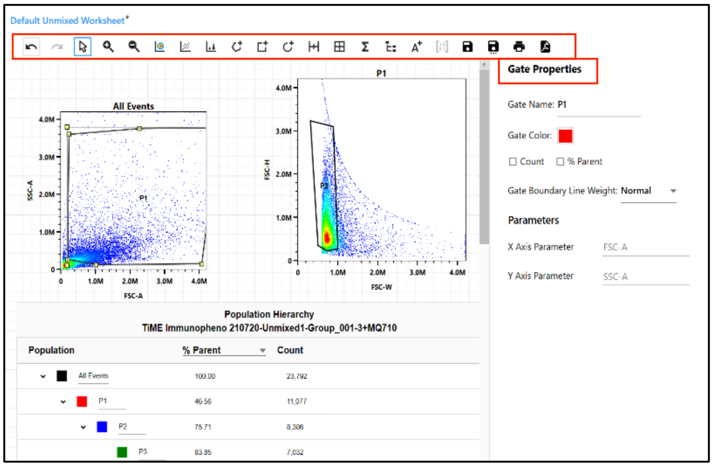


Name
Worksheet 52522

Description (optional)

Name	Created By	Type
Cytek 14C IP Panel	Admin	Unmixed

2) At this point you can start recording your experimental samples, edit the experimental setup, create plots, edit gates properties, and analyze your data.



Gate Name: P1

Gate Color: 🟥

Gate Boundary Line Weight: Normal

X Axis Parameter: FSC-A

Y Axis Parameter: SSC-A

Population Hierarchy
TiME Immunopheno 210720-Unmixed1-Group_001-3+MQ710

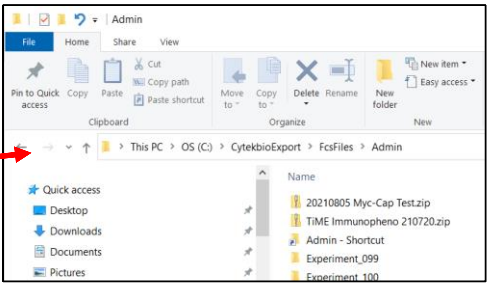
Population	% Parent	Count
■ All Events	100.00	23,792
🟥 P1	46.56	11,077
🟦 P2	75.71	8,386
🟩 P3	83.85	7,032

6. Data export

You have a few options while exporting your experimental data.

1) Exporting FCS files:

- FCS files (both RAW and Unmixed) are automatically saved once samples are recorded. FCS files can be found under:

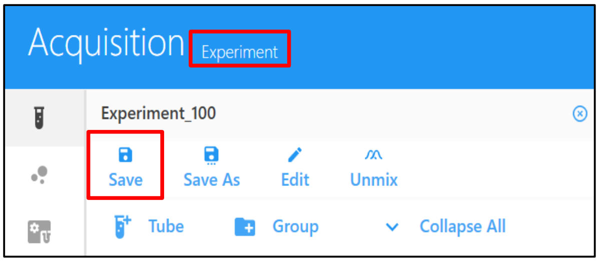


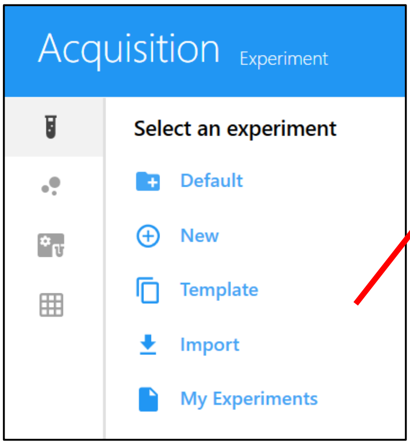
This PC > OS (C:) > CytekbioExport > FcsFiles > Admin

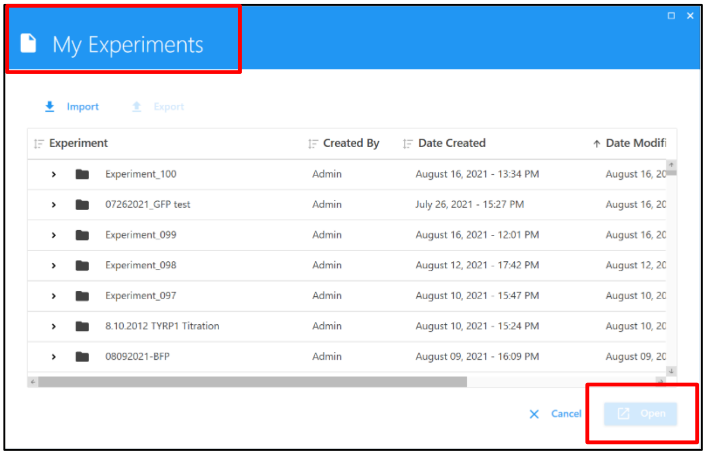
- 20210805 Myc-Cap Test.zip
- TiME Immunopheno 210720.zip
- Admin - Shortcut
- Experiment_099
- Experiment_100

2) Exporting entire experiment:

- In order to save entire experiment, click on icon **save.** This option allows to keep all the previously chosen settings together recorded data.
- Once saved, it can be found and re-imported from the Acquisition tab under “**my experiments**”.



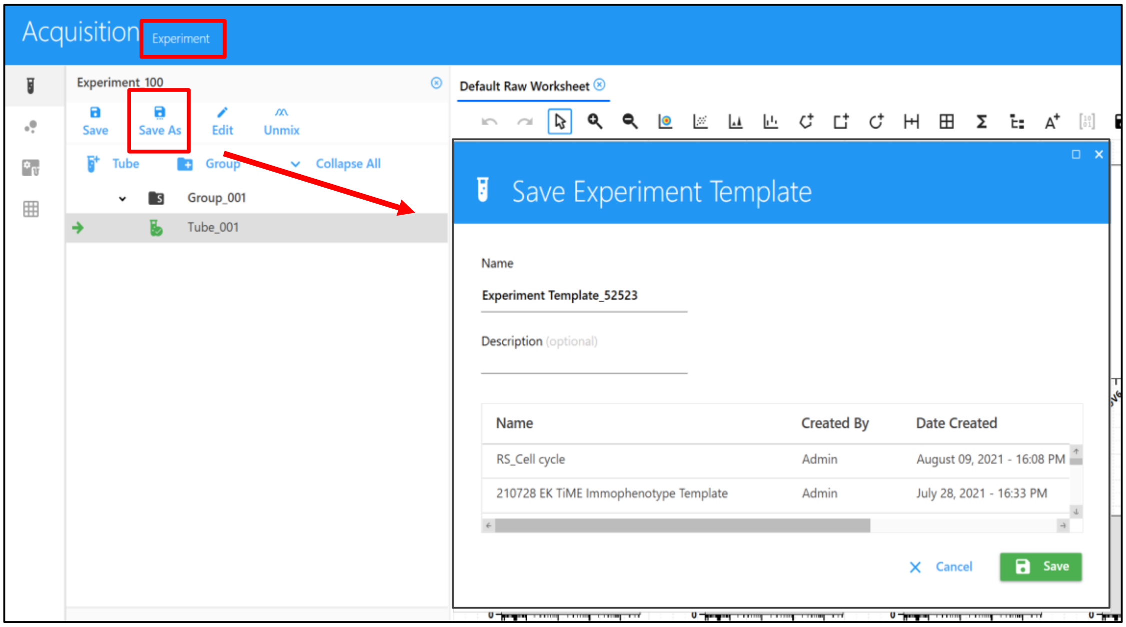




Experiment	Created By	Date Created	Date Modifi
Experiment_100	Admin	August 16, 2021 - 13:34 PM	August 16, 20
07262021_GFP test	Admin	July 26, 2021 - 15:27 PM	August 16, 20
Experiment_099	Admin	August 16, 2021 - 12:01 PM	August 16, 20
Experiment_098	Admin	August 12, 2021 - 17:42 PM	August 12, 20
Experiment_097	Admin	August 10, 2021 - 15:47 PM	August 10, 20
8.10.2012 TYRP1 Titration	Admin	August 10, 2021 - 15:24 PM	August 10, 20
08092021-BFP	Admin	August 09, 2021 - 16:09 PM	August 09, 20

3) Exporting template:

- In order to save the entire experiment template with all previously chosen reference controls, markers, keywords and recording options, you select from the experiment: save as.



Save Experiment Template

Name

Experiment Template_52523

Description (optional)

Name	Created By	Date Created
RS_Cell cycle	Admin	August 09, 2021 - 16:08 PM
210728 EK TiME Immophenotype Template	Admin	July 28, 2021 - 16:33 PM

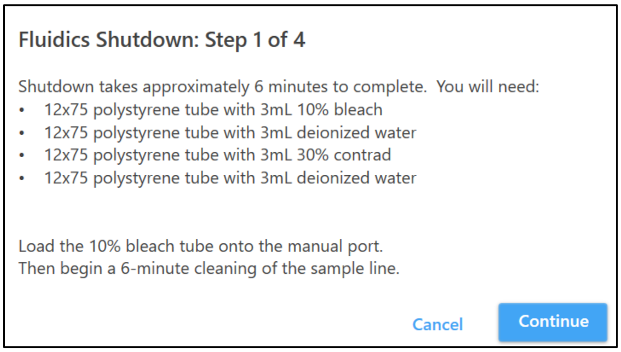
7. Cleaning

1) Clean the analyzer after your experiment is finished. Go to the **Acquisition module**, select Fluidics Shutdown, and follow on-screen prompts.

- If running the plate loader, clean the plate loader first as referenced in the Aurora Plate Loader Reference Guide and then proceed with Fluidics Shutdown cleaning.

2) Prepare tubes containing:

- 3 mL of 10% bleach
- 3 mL of DI water
- 3 mL of 30% Contrad 70
- 3 mL of DI water



Fluidics Shutdown: Step 1 of 4

Shutdown takes approximately 6 minutes to complete. You will need:

- 12x75 polystyrene tube with 3mL 10% bleach
- 12x75 polystyrene tube with 3mL deionized water
- 12x75 polystyrene tube with 3mL 30% contrad
- 12x75 polystyrene tube with 3mL deionized water

Load the 10% bleach tube onto the manual port.
Then begin a 6-minute cleaning of the sample line.

3) In the last step of Fluidics Shutdown's procedure **don't turn off the cytometer unless you are the last user of the day.**

Note 2: the default mode is tube. Therefore, if plate mode is used, the instrument must be put back in tube mode for next user, unless it is confirmed the next user will also be using plate mode.